Towards an understanding of membrane-mediated protein–protein interactions

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We propose a computational framework to study the lipid-mediated clustering of integral membrane proteins. Our method employs a hierarchical approach. The potential of mean force (PMF) of two interacting proteins is computed under a coarse-grained 3-D model that successfully describes the structural properties of reconstituted lipid bilayers of dymiristoylphophatidylcholine (DMPC) molecules. Subsequently, a 2-D model is adopted, where proteins represented as self-avoiding disks interact through the previously computed PMF, which is modified to take into account three body corrections. The aggregation of the proteins is extensively studied under the condition of negative hydrophobic mismatch: the formation of clusters with increasing size agrees with previous computational and experimental findings.

1 Introduction

Biomembranes and membrane proteins are fundamental for the physiology of the cells:[1] lipid-mediated interactions among embedded proteins might form clusters, which are crucial for performing vital biological processes occurring in living cells.[2,3] There are different types of membrane proteins, which all interact in specific ways among each other within the membrane environment.[4,5] Nonetheless, a quite general characterization of their interaction is made possible by a structural property called hydrophobic mismatch,[4,5] which is the difference between the hydrophobic lengths of the protein and the lipid bilayer. Modulations of the bilayer thickness, protein tilting, protein functioning and protein aggregation have been shown experimentally to depend strongly on the protein hydrophobic mismatch.

Several theoretical studies have been published which address the interaction among membrane proteins.[6–10] Molecular simulations of lipid bilayers are valuable for providing insights into the microscopic structure of reconstituted membrane systems.[11–18] The limit in length and time scales afforded by such approaches restricts the number of proteins that might be simulated, and thus hinders the study of membrane protein clustering. Three-dimensional coarse-graining techniques allow simulators to bridge the gap between atomistic and phenomenological descriptions of complex systems and are therefore optimal candidates for an integrated study on

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biological membranes.[19–21] Recently, three-dimensional coarse-grained models and the dissipative particle dynamics (DPD) simulation technique were used to systematically compute the potential of mean force (PMF) between two proteins as a function of the hydrophobic mismatch of the proteins.[22,23] Experimentally, it is difficult to determine the size of the protein clusters in a biological membrane.[4,5] Recent results indicate that the average size of the observed protein clusters could be of the order of 50–100 proteins,[4] and even much higher.[24] Simulating such a large number of proteins is, even for the three-dimensional coarse-grained models, very demanding.

In the present study we explore a hierarchical coarse-grained framework to study membrane-mediated protein clustering. Conventional Monte Carlo simulation in an NVT ensemble[25] is used to study a two-dimensional model in which the proteins interact through effective potentials that are based on the potentials of mean force (PMF) computed using our three-dimensional coarse-grained model.

Our goal is to obtain a better understanding of the lipid-mediated interactions between membrane proteins. In particular, we address the question whether the clustering of membrane proteins can be simulated using the computed PMF as an effective pair potential, which includes a first-order approximation of three-body effects. We illustrate our approach by studying the clustering behavior of a model protein with negative mismatch. The model protein could represent gramicidin, which is well known to cluster as a result of the negative mismatch when embedded in a dymiristoylphophatidylcholine (DMPC) bilayer.

2 Mesoscopic model and simulation details

In this work we introduce two hierarchically connected coarse-grained models. The starting point is a mesoscopic model in which groups of atoms are lumped into a pseudo particle (see Fig. 1). The effect of water is explicitly modeled; three water atoms are regrouped into one water bead. The key aspect is that hydrophilic and hydrophobic interactions are described in terms of differences in repulsive interactions.[26,27] For example, moving a hydrophilic particle from a hydrophobic environment towards a hydrophilic one reduces the net repulsion and hence lowers the total energy of the systems. The parameters of this soft-repulsive interactions model have been obtained from solubility parameters.[28,29] The intramolecular potentials that connect the pseudo atoms of the lipid have been obtained from fitting to all-atom

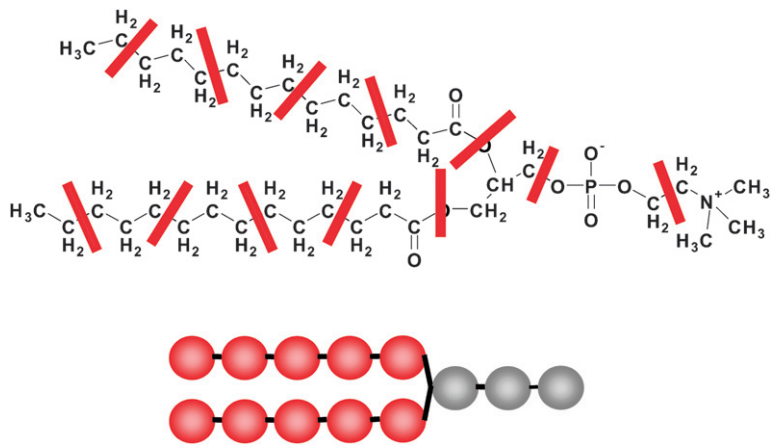

Fig. 1 Coarse-grained dimiristoylphosphatidylcholine (DMPC). We assume that the volume of a coarse-grained particle is approximately 90 Å^3 and with this assumption we map the all-atom representation onto the coarse grained particles.

simulations of a single phospholipid in water.[30] The model lipid that is used in the current study is coarse-grained DMPC. Previous computational studies have shown that this lipid model forms a stable bilayer and displays the typical temperature phase behavior of lipid bilayers.[31]

For the membrane proteins, we focus on the effect of the hydrophobic mismatch. A protein is considered as a rod-like object (see Fig 2). The top and the bottom part of the rod are hydrophilic and the middle hydrophobic. Transmembrane proteins are built by connecting hydrophobic-like beads into a chain and attaching to the ends hydrophilic groups. These chains are then linked together into a bundle of N_P of these amphipathic chains. In each model protein, all the N_P chains are linked to the neighboring ones by springs, to form a relatively rigid body. The diameter of the protein can be changed using different values of N_P. For example, a protein, which mimics the shape[32] of an alpha helix (such as gramicidin A) is constructed by a central chain surrounded by a single layer of six other chains. We denote such a protein by $N_P = 7$. The hydrophobic thickness of the protein can be adjusted by changing the number of hydrophobic beads. The hydrophobic mismatch is defined as the difference between the hydrophobic length of the protein core and the hydrophobic thickness of the pure lipid bilayer. As a consequence, in our model the system can respond to accommodate the hydrophobic mismatch by tilting or changing the thickness of the membrane. In our model the proteins do not have appreciable internal flexibility. We therefore do not allow the proteins to change conformation except for bending. We observe some small bending for positive mismatch and small diameters, but, not for large diameters, because of geometric reasons. However, as we did not optimize these parameters to represent a realistic flexibility of particular proteins, we focus on those systems for which this bending effect is small. Details of the model and the parameters can be found in the literature.[26,30,33]

We used dissipative particle dynamics[29] (DPD) to simulate the properties of our mesoscopic model. The equations of motion were integrated using a modified version of the velocity Verlet algorithm with a reduced timestep of 0.03. The main modification of the standard DPD algorithm is a method we have implemented to ensure that the membrane is simulated in a tensionless state. After on average 15 timesteps a Monte Carlo step was made which involved an attempt to change the area of the lipid in such a way that the total volume remained constant. The acceptance rule for this move involves the imposed interfacial tension,[34] which was set to zero for our simulations. To ensure sufficient hydration, we used a system of 100 000 water molecules for a total of 4,000 lipid molecules.

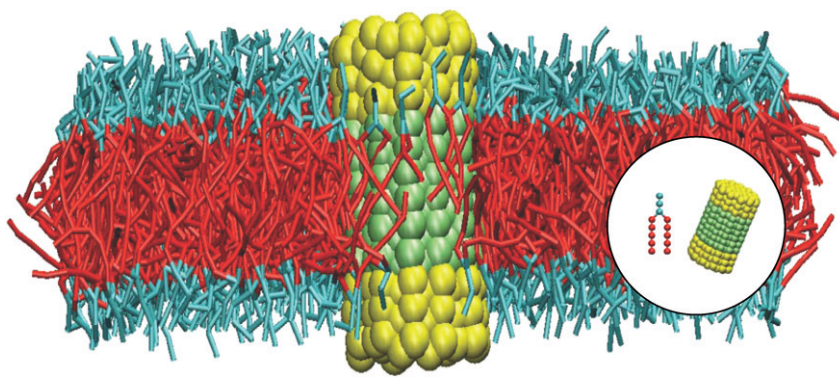

Fig. 2 Schematic representation of model lipids and transmembrane protein.

3 Potential of mean force

The second model that we introduce in this work uses as input the potential of mean force (PMF), which is defined as the reversible work needed to bring two proteins from infinity to a given distance. Before introducing this model we discuss some typical potentials of mean force that we have obtained for this system. Fig. 3 shows some typical potentials of mean force for different values of the hydrophobic mismatch. For negligible mismatch we do not observe any clustering and the PMF is essentially zero. Fig. 3 shows that for large negative and large positive mismatches, however, we see a long-range attraction between the proteins. At this point it is important to emphasize that in our model the intermolecular interactions are short-ranged repulsive. As a consequence, the observed long-range interactions are caused by the perturbation of the membrane as a result of the insertion of the proteins. Indeed, both for a negative and positive mismatch, the membrane around the protein has to change its thickness to accommodate the hydrophobic mismatch. If the proteins cluster, then the total perturbation is less than if the proteins are infinitely far apart. A difference between negative and positive mismatch is found in the range of the interactions. For positive mismatch this range is much shorter.

It is instructive to compare these results with the recent simulations of Schmidt *et al.*,[22] who obtained a potential of mean force that is very different. The results of Schmidt *et al.* suggest that a large energy barrier is keeping two proteins together in a membrane. If we compare our potential of mean force calculations with the theoretical predictions of Dan *et al.*[8] and Kralchevsky *et al.*,[9] our conclusions are the opposite of Schmidt *et al.* We do not observe the high energy barrier observed in the calculations of Dan *et al.* As pointed out by Kralchevsky *et al.*, in the case of zero surface tension, which is imposed in our simulations, the theory of Dan *et al.* should be very similar to the theory of Kralchevsky *et al.* In fact, depending on the choice of parameters, a repulsive barrier can be the result of the model of Kralchevsky *et al.*, if the lipid profile in between the two proteins differs very much from the single protein profile. Our results are in nice agreement with the calculations of Bohinc *et al.*[35] However, in these theories it is assumed that the proteins do not tilt, which is a good approximation for proteins with a large diameter,[33] but may not hold for proteins with a small diameter.

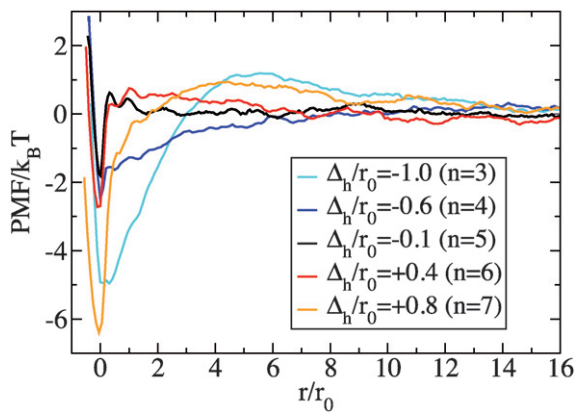

Fig. 3 Potential of mean force as a function of the distance between two proteins with negative ($\Delta_h/r_0 = -1.0$ and -0.6), negligible ($\Delta_h/r_0 = -0.1$), and positive ($\Delta_h/r_0 = +0.4$ and $+0.8$) mismatch. The mismatch is defined as $\Delta_h = h - h_0$, with h the bilayer thickness at the surface of the protein and h_0 the unperturbed bilayer thickness. The mismatch is in units of r_0, which is the interaction cut-off diameter, $r_0 = 6.46$ Å.

Two-dimensional model

Compared to all-atom simulations our mesoscopic model reduces the required amount of CPU time significantly. Extending this model, however, to a very large number of proteins that would allow us to study the clustering behavior would still lead to prohibitively large CPU requirements. Therefore we introduced a two-dimensional model in which the lipids are described as an implicit medium. The interactions between our two dimensional proteins are obtained from the potential of mean force of the mesoscopic model.

Goldman *et al.*[36] have studied the clustering of membrane proteins using a two-dimensional model. In their approach the proteins are modeled as lattice sites having only nearest-neighbor interactions. Implicit in this model is the assumption that protein–protein interactions can be described with a simple pairwise-additive potential. In this work we investigate this assumption in detail and we show that only under negligible hydrophobic mismatch is this assumption reasonable. For any sizeable hydrophobic mismatch, three-body interactions among embedded proteins cannot be neglected, even at low protein concentrations.

To quantify the effect of many body interactions, we compute the PMFs for a protein approaching a cluster of two (Fig 4a) and a cluster of seven proteins (Fig 4b). The hydrophobic mismatch is $(h - h_0)/r_0 = -1$, where r_0 is the cut-off radius of the potential, and h and h_0 the hydrophobic thickness of the protein and the membrane, respectively. For this particular mismatch the attractive forces between the proteins are sufficiently large to compel those proteins that are part of the cluster to remain in the cluster during the entire simulation. All simulations were at the reduced temperature, $T = 0.7$, which corresponds to approximately 60 °C, high enough to ensure that the bilayer is in the fluid phase.[33]

Fig. 4a compares the PMF as obtained from the mesoscopic simulations, with the results from a pairwise two-dimensional model. In this two-dimensional model, in which the proteins are modeled as two-dimensional disks, the intermolecular potential is the PMF as obtained from the mesoscopic simulations for two proteins (*e.g.* see Fig. 3). If we assume pairwise-additive interactions, we can compute the energy of the three proteins cluster. This energy depends on the details of the trimer configuration. If we compute the energy along the dimer axis ($\theta = 0$), we find a lower energy for large distances compared to a perpendicular ($\theta = 90$), while at short distances the triangular

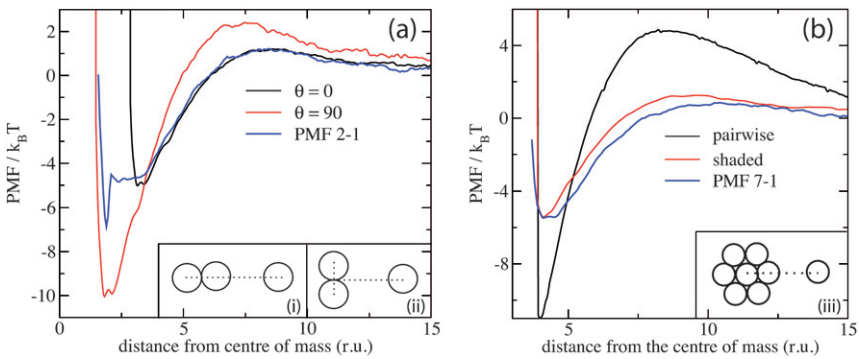

Fig. 4 Potential of mean force (PMF) as a function of the distance between a cluster of proteins and a single protein. Figure (a) shows a cluster of two proteins and figure (b) shows a cluster of seven. (a): PMF 2-1 is computed with the 3-D model the other curves are computed using the pairwise 2-D model in which we use two angles of approach, θ; the black line is for $\theta = 0$, see inset (i), and red for $\theta = 90$, see inset (ii). Right, (b): PMF 7-1 is computed using the 3-D model, the other two lines for the pairwise-additive model and the shaded 2-D model. In both plots, the distance between the proteins is in reduced units (1 r.u. = 6.46 Å). In the two-dimensional model a cut-off radius of around 18 r.u. was used.

configuration has the lowest energy. If we compare these results with the PMF of the mesoscopic model, we see that at large distances there is good agreement with the $\theta = 0$ approach, which is indeed the cluster orientation that has the dominant contribution in the PMF. For short distances the triangular, ($\theta = 90$), orientations are dominant in the PMF. For this case, however, the pairwise-interaction potential in our two-dimensional model overestimates the net attractive interactions. As in our system the mechanism of protein association is the perturbation of the membrane, a pair potential overestimates the total perturbation. Fig. 4b illustrates another situation in which the pairwise-additive potential fails to correctly describe the interactions. Clearly, in a cluster of 7 proteins the middle protein is completely screened from the membrane and therefore does not contribute to the total interactions; as a consequence, the pairwise-additive model overestimates the extent of the repulsive and attractive interactions.

A more realistic description of our two-dimensional model is to introduce three body interactions that take into account that the presence of a third protein screens the interactions with the membrane. For this we introduce a screening parameter for a particular interaction. Let us assume we have three proteins: i, j, and k (see Fig 5). We first compute the polar angles φ_1 and φ_2 of the centers of mass of i and j, respectively, and the angles θ_1 and θ_2 defined by tangents to i and j from the center of k. A protein is denoted shaded by another one, if the following criterion holds: $(\theta_1 + \theta_2)/2 < |\varphi_1 - \varphi_2|$, where φ_1 and $\varphi_2 \in [-\pi, \pi]$, as shown in Fig. 5. Each protein interacts only with non-shaded ones and does not feel the presence of the shaded proteins. The first neighbor of each protein cannot be shaded, while for all the rest, the above criterion determines whether they are shaded by another protein or not. Fig. 4b shows that if we compute the energy of our two-dimensional system by summing over all non-shaded pairs, we obtain a much better description of the PMF between a cluster of seven and a single protein. This comparison indicates that we have captured in our model the most important contribution of the three-body interactions. A further improvement of this model would be the correction for the double counting of the perturbation in case of the triangular orientation.

We use the screened two-dimensional model to study the clustering. We observe that even at low densities (100 proteins with 0.001 proteins per unit area) all the proteins aggregate and finally form one big cluster, while splitting in smaller aggregates is not observed. Single proteins do continue to escape the cluster and later merge again. It is interesting to contrast these results with our pairwise-additive

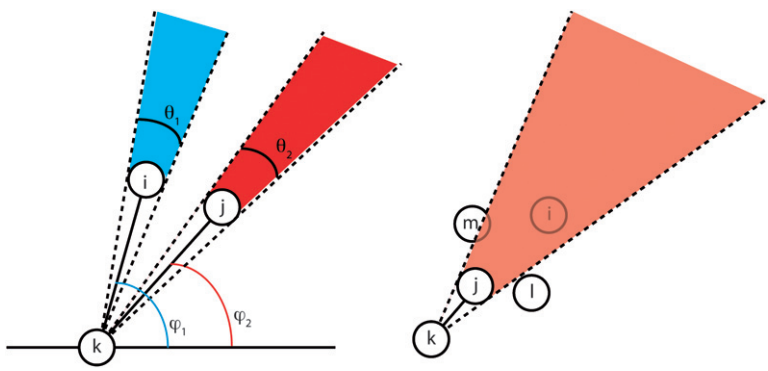

Fig. 5 (Left) Illustration of the screening in the 2-D model: the particles i and j screen all particles in the shaded areas from interacting with particle k. (Right) Because of the screening by protein j, protein k interacts only with j and l.

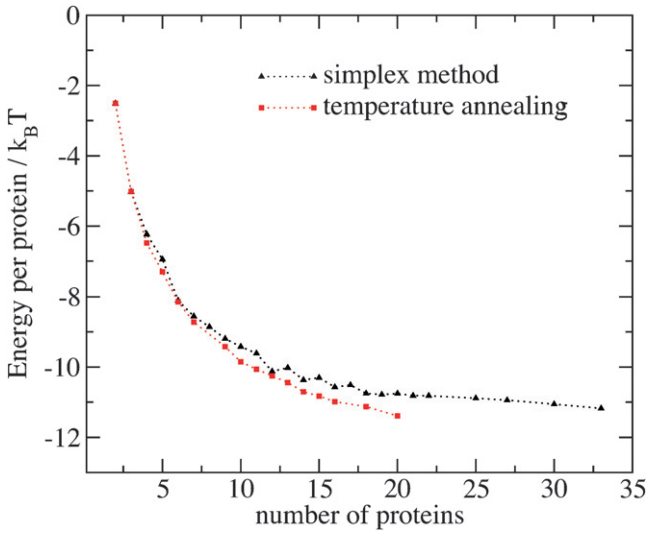

Fig. 6 Ground state energies per protein of the system with negative hydrophobic mismatch are computed using both the simplex method and simulated annealing. The latter is computationally more demanding and it was not easy to obtain reliable minima for systems with more than 20 proteins. Otherwise the two methods show a good agreement.

model. For this system we find a well-defined cluster distribution, which is caused by the repulsive barrier shown in Fig 4b.

To have a better understanding whether phase separation of proteins from the lipids is favored (protein enriched phase), we have studied the ground state configurations of systems of up to 35 proteins. We focused on the question of whether there is a critical cluster size for which the energy is minimal: this would indicate that for any temperature, phase separation would be entropically disfavored and clustering of proteins would take place with an upper limit of the cluster size.[37,38] Searching the ground state of a rugged potential energy surface is a hard task, for which a general solution in a short time scale is not always feasible. Furthermore, there is no way to prove that the ground state is unique.[39] Due to this, we have tried obtaining the ground states of the systems studied here using two different methods: the simplex method[39] and temperature annealing.[40] Fig. 6 shows that with both methods, as the number of proteins in the cluster increases, the energy per protein continues to decrease. This indicates that for systems with comparable densities and number of proteins (*i.e.* up to 100) aggregation, or phase separation, will be favored rather than the formation of a number of clusters.

These observations are in agreement with the known properties of gramicidin within phospholipid bilayers: in fact, previous experimental studies[41,42] have shown the tendency of gramicidin to form big aggregates even in the gel phase. This behavior has been confirmed as expected in the fluid phase, both by computer simulations of elastic models of reconstituted lipid bilayers[6,7,10] and by experiments,[24] notwithstanding the difficulty to detect the tiny mismatch between the lipids in the fluid phase and gramicidin clusters by atomic force microscopy.

Concluding remarks

The methodology developed and used in this study allows us to obtain a better understanding of membrane-mediated interactions. This work illustrates that the protein–protein interactions cannot be described as pairwise additive and that

even at low densities three-body interactions are important. We have made the first step in developing such an effective three-body potential, which allows us to extend these calculations to very large systems.

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